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Loss of Cardioprotective Effects at the ADAMTS7 Locus as a Result of Gene-Smoking Interactions

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1 Loss of cardio-protective effects at the *ADAMTS7* locus due to gene-smoking interactions

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21 Running Title: Gene*Smoking interaction, *ADAMTS7* locus & CHD risk

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138 **ABSTRACT**

139 **Background** Common diseases such as coronary heart disease (CHD) are complex in etiology. The
140 interaction of genetic susceptibility with lifestyle factors may play a prominent role. However, gene-
141 environment interactions for CHD have been difficult to identify. Here, we investigate interaction of
142 smoking behavior, a potent lifestyle factor, with genotypes that have been shown to associate with
143 CHD risk.

144 **Methods** We analyzed data on 60,919 CHD cases and 80,243 controls from 29 studies for gene-
145 smoking interactions for genetic variants at 45 loci previously reported to associate with CHD risk.
146 We also studied 5 loci associated with smoking behavior. Study specific gene-smoking interaction
147 effects were calculated and pooled using fixed-effects meta-analyses. Interaction analyses were
148 declared to be significant at a $P\text{-value} < 1.0 \times 10^{-3}$ (Bonferroni correction for 50 tests).

149 **Results** We identified novel gene-smoking interaction for a variant upstream of the *ADAMTS7* gene.
150 Every T allele of rs7178051 was associated with lower CHD risk by 12% in never-smokers ($P\text{-value}$:
151 1.3×10^{-16}) compared to 5% in ever-smokers ($P\text{-value}$: 2.5×10^{-4}) translating to a 60% loss of CHD
152 protection conferred by this allelic variation in people who smoked tobacco (*Interaction $P\text{-value}$* :
153 8.7×10^{-5}). The protective T allele at rs7178051 was also associated with reduced *ADAMTS7*
154 expression in human aortic endothelial cells and lymphoblastoid cell lines. Exposure of human
155 coronary artery smooth muscle cells to cigarette smoke extract led to induction of *ADAMTS7*.

156 **Conclusion** Allelic variation at rs7178051 that associates with reduced *ADAMTS7* expression
157 confers stronger CHD protection in “never-smokers” compared to “ever-smokers”. Increased
158 vascular *ADAMTS7* expression may contribute to the loss of CHD protection in smokers.

159 **Key words:** Gene-smoking interaction, gene-environment interaction, coronary heart disease,
160 *ADAMTS7*, smoking.

161 **Word count: 269**

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164 Clinical Perspective

165 1) What is new?

- 166
- 167 • Using data on 60,919 CHD cases and 80,243 controls, this study conducted gene-
168 environment interaction analyses to investigate effect modification by smoking behavior at
169 established CHD and smoking related loci.
- 170 • Cardio-protective effects associated with allelic variation at the *ADAMTS7* locus were
171 attenuated by 60% in people who smoked tobacco compared to those who did not smoke.
- 172 • Allelic variation at *ADAMTS7* associated with reduced CHD risk was associated with reduced
173 *ADAMTS7* expression in human aortic endothelial cells and lymphoblastoid cell lines.
- 174 • Exposure of human coronary artery smooth muscle cells to cigarette smoke extract led to
175 induction of *ADAMTS7*.

176

177 2) What are the clinical implications?

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- 179 • These human genomic data provide new insights into potential mechanisms of CHD in
180 cigarette smokers.
- 181 • Findings from this study also point towards the directional impact of the *ADAMTS7* locus on
182 CHD.
- 183 • *ADAMTS7* and its substrates have a specific role in cigarette smoking related CHD.
- 184 • Inhibition of *ADAMTS7* is a novel potential therapeutic strategy for CHD that may have
185 particular benefits in individuals who smoke cigarettes.

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194 INTRODUCTION

195 Coronary heart disease (CHD) is a complex disorder resulting from the interplay of lifestyle
196 and genetic factors.^{1, 2} Yet, gene-environment interactions for CHD have been difficult to identify.
197 Cigarette smoking is one of the strongest lifestyle risk factors for CHD but the underlying molecular
198 mechanisms of CHD in humans who smoke remain uncertain.³⁻⁵ Cigarette smoking accounts for
199 one-fifth of all CHD events globally and is responsible for ~1.6 million deaths attributable to CHD
200 annually.⁶ Genome-wide association studies (GWAS) have improved our understanding on the
201 genetic predisposition to both CHD and smoking behavior.⁷⁻¹⁰ Joint or interactive effects of genetic
202 variation and smoking behavior in the etiology of CHD, however, remain poorly understood. GWAS
203 can provide new opportunities to investigate gene-smoking interactions.

204 We hypothesized that genetic predisposition to CHD is modified by cigarette smoking at
205 loci discovered by GWAS to be associated with either CHD or smoking behavior. We conducted a
206 focused experiment at 50 main-effect loci (45 for CHD and 5 for smoking behavior) using genetic
207 data and information on smoking behavior in 60,919 CHD cases and 80,243 controls from 29
208 different studies. We report novel findings on gene-smoking interactions in CHD.

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221 **METHODS**

222 **Summary of study Design**

223 All studies participating in the CARDIoGRAMplusC4D consortium⁷⁻⁹ that had information
224 available on smoking status, CHD risk and genotypes at the 50 CHD and smoking behavior-
225 associated loci were invited to participate. The current study had five inter-related components
226 (**Supplementary Figure-1**). First, as part of the quality control, we investigated the association of
227 smoking status with CHD risk within each study. Second, we performed an updated analysis of all
228 the SNPs (\pm 50 KB) at the 45 established CHD loci to identify the variant with the strongest CHD
229 association in our study population at each established CHD locus. Effect estimates from each study
230 in association with CHD risk were obtained and pooled to identify the strongest CHD associated
231 variant (“lead variant”). Third, we investigated gene-smoking interactions at these 45 CHD loci and at
232 5 loci related to smoking behavior. Fourth, for loci demonstrating differential CHD associations by
233 smoking status, we mapped the interaction region, examined linkage disequilibrium (LD) across the
234 region and performed conditional analyses to identify independent genetic signals. Finally, for loci
235 exhibiting gene-smoking interaction in CHD, we assessed eQTL data for association of variants with
236 expression of local genes in available datasets and examined expression of these genes in multiple
237 cell types that play prominent roles in smoking-CHD pathobiology.

238 **Harmonization of phenotypes and genotypes**

239 Summary level estimates for each study were shared via a secure FTP site. We used
240 “ever-smoking” as a primary exposure and data were harmonized by uniformly characterizing
241 participants in each study into two categories, “ever-smokers” and “never-smokers”. “Ever-smokers”
242 were defined as those who had smoked more than 100 cigarettes in a lifetime. For case-control
243 studies, information on “ever smoking” status collected at the time of enrollment was used for the
244 current analyses; whereas for prospective cohort studies, information on smoking status obtained at
245 the baseline visit was used for the current investigation. CHD was defined based on evidence from
246 angiography or history of verified myocardial infarction (MI), percutaneous coronary interventions
247 (PCI) or coronary artery bypass grafting (CABG) as published in CARDIoGRAMplusC4D projects.⁷⁻⁹
248 Genotype data generated through GWAS (directly genotyped or imputed) or cardio-metabochip
249 (directly genotyped only) arrays were obtained from each study and all genetic data were aligned
250 using the build-37 reference panel. Imputed SNPs were removed if they had any of the following: (i)
251 a minor allele frequency of $<1\%$; (ii) info score of <0.90 ; or (iii) confidence score <0.90 . For each
252 study, GWAS data were imputed using the Phase II CEU HapMap reference population.¹¹ Standard

quality control criteria were applied by each participating study, as described previously.⁷ All participating studies in the CARDIoGRAMplusC4D consortium were approved by their locally relevant institutional review boards and all participants gave written informed consent before their enrollment in each study.⁷⁻⁹

STATISTICAL ANALYSIS

Gene-smoking interaction analyses

Initial quality control and association of established CHD loci with CHD risk: As part of an initial quality control, effect estimates from each study were obtained for “ever-smoking” status and CHD risk using a case-control logistic regression model adjusted for age and sex. Each participating study also assessed and, if needed, controlled for population stratification by including principal components as covariates in the regression model as described earlier.⁷⁻⁹ To identify variant(s) with the most significant association with CHD risk at established CHD loci in our study population, logistic regression analyses were conducted by each participating study for all the SNPs flanking (± 50 kb) the lead variant previously reported at each CHD locus. Effect estimates and standard errors were obtained and meta-analyzed using a fixed-effects inverse variance approach. All lead variants identified through these analyses were further investigated for gene-smoking interactions in CHD. One lead variant per locus was selected for primary gene-smoking interaction analyses.

Investigation of the APOE locus: Although APOE has been recently established as a GWAS locus,⁷ previous studies prior to GWAS have suggested that CHD risk is higher among carriers of the $\epsilon 4$ allele at the APOE locus in smokers than in non-smokers.¹²⁻¹⁴ Because the $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles at the APOE locus are not captured by the GWAS platform, we specifically conducted genotyping for rs429358 and rs7412 variants to capture the three epsilon (ϵ) alleles in 13,822 participants (including 7,286 first-onset myocardial infarction cases) in the PROMIS study.¹⁵

Gene-smoking interaction analyses at CHD and smoking loci: To assess gene-smoking interactions, analyses were conducted within each study, adjusted for age, sex and other study specific covariates (e.g., principal components), and variants were analyzed in association with CHD separately in “ever-smokers” and “never-smokers”. Results from the two groups were then used to test for interaction within each study. For the 50 variants, an interaction test statistic was calculated within each study using the following equation as adapted from Teslovich *TM* et.al.¹⁶

$$\frac{(\beta_n - \beta_e)}{\sqrt{SE_n^2 + SE_e^2}}$$

283 where β_n and β_e are the beta coefficients for the SNP in never-smokers and ever-smokers
284 respectively, SE_n and SE_e are the standard errors for the log-ORs estimated for never-smokers and
285 ever-smokers, respectively. Study specific interaction beta(s) and se(s) were calculated within each
286 study and were pooled across studies using a fixed-effects meta-analysis. Interaction analyses were
287 declared to be significant at a P-value of $<1.0 \times 10^{-3}$ (Bonferroni correction for 50 tests).

288 Conditional analyses on chr.15q25.1: At chr.15q25.1, we observed two variants exhibiting gene-
289 smoking interactions for CHD. The proximity of these two signals raised the possibility that the
290 observed interactions may represent a single interaction locus across the entire region. To
291 investigate this possibility we undertook conditional analyses using an approximate conditional and
292 joint analyses approach, also known as GCTA (Genome-wide Complex Trait Analysis), as described
293 previously.¹⁷⁻²² Briefly, this method leverages summary-level statistics from a meta-analysis and uses
294 LD corrections between SNPs estimated from a reference sample. Such an approach has been
295 shown to yield similar results to that obtained from conditional analyses conducted on individual
296 participant data and has been successfully implemented in several other studies that have fine-
297 mapped loci for other complex traits.¹⁷⁻²² Using this approach, we first conducted separate
298 conditional analyses at the chr.15q25.1 locus to identify main-effect variant(s) independently
299 associated with CHD and smoking behavior, respectively. We used the meta-analyzed data for CHD
300 main effects in the CARDIoGRAMplus4D consortium to identify SNPs independently associated with
301 CHD risk and we used the genetic meta-analysis data from the Tobacco and Genetics Consortium
302 (TGC) in 140,000 participants to identify variants independently associated with smoking behavior.
303 We then estimated the effects of these independent variants on CHD risk stratified by smoking
304 status and mutually adjusted the effects of these variants for each other.

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306 **Analysis of eQTLs and regulatory features at the chr15q25.1 gene-smoking interaction locus**

307 eQTL analyses: We mined publicly available databases to identify genotype-related expression
308 differences (eQTLs) in *ADAMTS7* and the *CHRNA3-A5* gene cluster in order to understand the
309 directionality of the association of expression of these genes with CHD and smoking behavior.
310 Specifically, we investigated data available from the GTEx consortium,²³ the HapMap consortium
311 (restricted to European populations), and the Multiple Tissue Human Expression Resource
312 (MuTHER).²⁴ We also analyzed expression data in 147 donor HAOEC lines.²⁵ We used a nominal P-
313 value of 0.002 to account for multiple testing involved in the eQTL analyses.

314 Regulatory features of the chr. 15q25.1 region: Data from ENCODE²⁶ were explored as described in
315 eMethods. ChIP-seq experiments were performed on confluent HCASMC (Cell Applications 350-05a
316 & Lonza CC-2583; cultured in SmGM-2 BulletKit media; Lonza) as described.²⁷ TCF21 (Abcam
317 ab49475), Jun (Santa Cruz Biotechnology sc-1694), JunD (Santa Cruz Biotechnology sc-74), and
318 CEBP (Santa Cruz Biotechnology sc-150) transcription factor binding was interrogated and H3K27ac
319 data were acquired using the same ChIP protocol with an anti-H3K27ac antibody (Abcam; ab4729).
320 Reads were aligned to the human genome (GRCh37p13) using STAR.²⁸

321

322 ***Analyses of ADAMTS7 and CHRNA3-A5 gene expression in vascular cells and tissues***

323 ADAMTS7 and CHRNA3-A5 gene expression in vascular cells: *ADAMTS7* and *CHRNA3-A5*
324 mRNA levels were measured in cultured human coronary artery smooth muscle cells (HCASMC;
325 Lonza CC-2583, Lonza Walkersville, MD), human coronary artery endothelial cells (HCAEC, Lonza
326 CC-2585), human aortic smooth muscle cells (HAoSMC, Lonza CC-2571), human aortic endothelial
327 cells (HAoEC, Lonza CC-2535), human aortic adventitial fibroblasts (HAoAF, Lonza CC-7014), and
328 human acute monocytic leukemia cell line (THP-1, ATCC TIB-202). Further details are provided in
329 eMethods.

330 ADAMTS7 and CHRNA3-A5 gene expression in response to cigarette smoke extract: HCASMC
331 were grown to confluence and cigarette smoke extract experiments performed at passage-7.
332 Cigarette smoke extract was custom-prepared by Arista Laboratories (Richmond, VA). Briefly, the
333 condensate was generated by smoking Marlboro Red King Size Hard Pack cigarettes on an
334 analytical smoke machine under International Organization for Standardization smoking conditions.
335 The smoke condensate was collected on 92 mm filter pads and extracted from each pad in DMSO
336 by shaking to obtain a solution of ~20 mg/mL final concentration of the total particulate matter.
337 Serum starved (24 hrs) HCASMC were treated with 0.5% or 1.0% cigarette smoke extract (v/v) for 4,
338 12, and 24 hrs in serum reduced conditions (0.5% FBS in DMEM). Details on RNA preparation and
339 q-PCR are provided in **Supplementary Methods**.

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344 RESULTS

345 *Description of the participating studies*

346 Of the 37 studies participating in the CARDIoGRAMplusC4D consortium, information on
347 “ever-smoking” was available in 30 studies, yielding a total sample size of 60,919 CHD cases and
348 80,243 controls. All studies recruited participants of European ancestry, except PROMIS (South
349 Asian),¹⁵ LOLIPOP (South Asian)²⁹ and FGENTCARD (Lebanese).³⁰ Number of CHD cases and
350 controls and percentages that were “ever-smokers” are provided in **Supplementary Table 1**. As
351 expected, in all the participating studies, association of “ever-smoking” status with CHD risk was
352 directionally consistent with an increased risk of CHD (**Supplementary Figure 2**).

353 *New variants associated with CHD at established loci*

354 **Supplementary Figure 3** and **Supplementary Table 2** present effect estimates for the
355 association with CHD for (i) the most significant variant that we identified at known CHD loci in the
356 current CARDIoGRAMplusC4D consortium analysis as well as for (ii) the top SNP previously
357 reported at each of these established CHD loci. Of the 45 established CHD loci, we identified 32 for
358 which we discovered a more statistically significant SNP in association with CHD risk in our dataset
359 than the prior reported top variant. All of these 32 SNPs were in moderate to high LD ($r^2 > 0.6$) with
360 the previously published variants.⁷⁻⁹ In our primary gene-smoking interaction analyses, at each of the
361 CHD loci, we, therefore, used the SNP with the most significant CHD association (**Supplementary**
362 **Figure 3** and **Supplementary Table 2**). Because the smoking behavior phenotype (captured as
363 cigarettes per day [CPD]) was not available in all CARDIoGRAMplusC4D studies, we used the top
364 variant previously reported for CPD¹⁰ at each locus (**Supplementary Figure 4**).

365 *Analyses of the APOE locus.*

366 The effect of rs6857, the lead CHD variant at the *APOE* locus, was similar in “ever-
367 smokers” compared to “never-smokers” (**Supplementary Table 3**). Specifically, the CHD OR for the
368 T allele at rs6857 was found to be 1.10 (P-value 7.93×10^{-4}) in “never-smokers” (12,159 CHD cases
369 and 22,932 controls) which was quantitatively similar to the CHD OR of 1.09 (P-value: 8.68×10^{-5})
370 observed in “ever-smokers” (23,753 CHD cases and 24,019 controls) (interaction P-value: 0.76)
371 (**Supplementary Figure 5a**). Investigation in the PROMIS study of the *APOE* epsilon genotypes
372 yielded consistent findings; the OR for CHD among $\epsilon 4$ carriers in “never-smokers” was 1.13
373 compared to the CHD OR of 1.07 observed in “ever-smokers” (interaction P-value: 0.82)
374 (**Supplementary Figure 5a**).

375 Novel gene-smoking interaction effects on CHD at chromosome 15q25.1

376 Of the 50 loci, we identified effect-modification by “ever-smoking” status on CHD risk for the
377 lead variants at two distinct loci, rs7178051, in proximity of *ADAMTS7* (an established CHD locus),
378 and rs1051730, in proximity of *CHRNA4-A3-A5* (an established smoking behavior locus)
379 (**Supplementary Table 3**). Although associated with different traits and located in distinct LD blocks,
380 these two variants reside ~224 KBs apart on chr.15q25.1 and are in weak linkage disequilibrium
381 (LD) ($r^2 = 0.22$), raising the question of whether these two chr.15q25.1 gene-smoking interactions on
382 CHD are independent of each other.

383 At the *ADAMTS7* CHD locus, the T allele at the rs7178051 variant was found to be more
384 strongly and inversely associated with CHD risk in never-smokers (OR: 0.88; P-value: 7.02×10^{-16})
385 compared to a much weaker effect in ever-smokers (OR: 0.95; P-value: 8.64×10^{-4}) (P-value of
386 interaction: 8.57×10^{-5}) (**Table 1**). Thus, the protective impact of the rs7178051 T allele observed in
387 never-smokers was halved in people who smoked (**Figure-1**). This difference is not related to power
388 differences within strata because for this variant, there were less data available in the never-smoking
389 group (21,232 CHD cases and 38,713 controls) compared to the ever-smoking group (39,585 CHD
390 cases and 40,749 controls). There was no substantial evidence of heterogeneity for the interaction
391 beta across the participating studies (Heterogeneity chi-squared = 36.23 (d.f. = 25); P-value for the
392 χ^2 test of heterogeneity = 0.06; $I^2 = 31.0\%$; tau-squared ($\tau^2 = 0$). We further conducted sensitivity
393 analyses using a random effect model; the results remained unchanged and the interaction beta
394 remained significant (**Supplementary Figure 5b**). Although the frequency of rs7178051 was 39% in
395 Europeans compared to 28% in South Asians, further analyses stratified by ancestry (i.e., European
396 versus non-Europeans) showed similar results (**Supplementary Figure 5c**). Other variants
397 discovered through prior CHD GWAS at the *ADAMTS7* locus (e.g., rs7173743, rs4380028,
398 rs3825807) were in moderate to high LD ($r^2 > 0.50$) with rs7178051 and were also found to display a
399 similar pattern of gene-smoking interaction effects (**Table 1**).

400 At the *CHRNA4-A3-A5* smoking locus, the A allele at the rs1051730 variant had an inverse
401 trend (not significant after adjustment) of association with CHD in never-smokers (OR: 0.96; P-value:
402 1.56×10^{-2}) and a positive trend (not significant after adjustment) in ever-smokers (OR: 1.03; P-value:
403 1.53×10^{-2}) (P-value of interaction: 2.37×10^{-4}) (**Table 1 and Supplementary Table 3**). For this
404 variant, data on 20,559 CHD cases and 38,198 controls were available in the never-smoking group
405 whereas 38,923 CHD cases and 40,371 controls were available in the ever-smoking group. Similar
406 gene-smoking interaction patterns were observed for other variants (e.g., rs2036527, rs8034191)
407 that have been previously reported for CPD behavior at the *CHRNA4-A3-A5* gene cluster (**Table 1**).

408 Further interrogation of the chr15q21.1 region encompassing rs7178051 and rs1051730
409 across three distinct LD blocks (**Figure 1**) revealed multiple additional variants for which we
410 observed gene-smoking interactions in CHD (**Table 1** and **Figure 1**). Indeed, several SNPs (e.g.,
411 rs7178051, rs10083696, rs7176187, rs6495335, rs4887077) had genome-wide significant
412 associations with CHD in “never-smokers” but had weaker and less significant associations with
413 CHD in “ever-smokers” (**Figure 1**). Alleles clustered specifically around *ADAMTS7* rather than at the
414 *CHRNA4-A3-A5* genes appear to be protective of CHD in “never-smokers” but have attenuated
415 protective effects in “ever-smokers” (**Figure 2**).

416 Conditional analyses

417 To investigate the possibility that the two chr.15q25.1 gene-smoking interactions might
418 represent a single interaction locus across the entire region we undertook an approximate
419 conditional and joint analyses¹⁷⁻²² using summary data derived from CARDIoGRAMplus4D for CHD
420 and from the TGC for smoking behavior. In-addition to rs7178051, we identified one other variant,
421 rs11072794 in low LD with rs7178051 ($r^2=0.20$) that was associated independently with CHD
422 (**Figure 3a**; red triangles) (**Figure 3b & Supplementary Figure 6b**; red triangles). We also
423 confirmed two variants (rs1051730 and rs684513) located in two different LD blocks that were
424 independently associated with smoking behavior in the TGC data¹⁰ (**Figure 3d & Supplementary**
425 **Figure 6b**; grey circles).

426 In analyses of the CHD variants, both rs7178051 and rs11072794 remained strongly
427 associated with CHD after adjusting for the top CPD variants (rs1051730 and rs684513) (**Figure 3d**,
428 red triangles) whereas their weak association with CPD was lost after adjusting for the top CPD
429 variants (**Figure 3d**; grey circles); e.g., the P-value for rs7178051 association with CPD was 1×10^{-5}
430 in unadjusted analyses but attenuated to 0.55 after adjusting for rs1051730 and rs684513. In
431 analyses of the CPD variants, both rs1051730 and rs684513 remained strongly associated with CPD
432 after adjusting for the top CHD variants (rs7178051 and rs11072794) (**Figure 3b**, grey circles)
433 whereas their weak association with CHD was lost after adjusting for the top CHD variants (**Figure**
434 **3b**, red triangles). As expected, conditional analyses that included all four of these variants resulted
435 in a null association of the region with both CHD and CPD (**Supplementary Figure 6b**). To
436 underscore the validity of the conditional approach using summary data, we used individual
437 participant data from an expanded PROMIS sample involving 9,025 MI cases and 8,506 controls.
438 We found that the OR conferred by allelic variation at rs7178051 remained associated with MI risk
439 independent of the two CPD variants (rs1051730 and rs684513) and rs11072794 (the second CHD

440 SNP) (**Supplementary Figure 6c**). Conversely, the apparent effect of allelic variation at rs1051730
441 (the top CPD variant) on CHD risk was lost when we adjusted for the other three variants,
442 rs7178051, rs11072794 and rs684513 (**Supplementary Figure 6c**).

443 Next, using summary level data we examined the association of each of these four variants
444 with CHD risk separately in “ever-smokers” and “never-smokers” while mutually adjusting for the
445 other three variants (**Figure 4 & Supplementary Figure 7**). In these analyses, only allelic variation
446 at rs7178051 was found to have independent genome-wide significant effects on CHD in never-
447 smokers. rs7178051 was also the only one of these four variants with significant differences in the
448 effect estimate for gene-CHD associations between the two smoking groups (P-value for the χ^2 test
449 of heterogeneity: 5.4×10^{-5}) after adjusting for the effects of other variants (rs11072794, rs1051730
450 and rs684513). These conditional analyses suggest that (a) variants located near the *ADAMTS7*
451 gene but not *CHRNA4-A3-A5* genes have independent effects on CHD, (b) a single independent
452 gene-smoking interaction signal for CHD exists on chr.15q.25.1 which is localized at the *ADAMTS7*
453 CHD locus (marked by rs7178051) and (c) an apparent interaction signal observed at the nearby
454 *CHRNA4-A3-A5* CPD locus (marked by rs1051730) is not independent of the *ADAMTS7*
455 (rs7178051) interaction signal.

456 To assess the robustness of conditional analyses methodology that uses summary level data
457 (i.e., GCTA)¹⁷⁻²², we conducted sensitivity analyses in the PROMIS dataset (9,025 MI cases and
458 8,506 controls). We assessed the association of rs7178051 (top CHD SNP) and rs1051730 (top
459 CPD SNP) after mutually adjusting for each other by conducting (i) standard logistic regression using
460 individual participant data and (ii) summary level data in PROMIS using the GCTA method
461 (**Supplementary Table 4**). The top CHD SNP was found associated with CHD risk in PROMIS
462 independent of the top CPD variant using both the methods, in-contrast the effect on CHD of the top
463 CPD SNP attenuated sharply when adjusted for the top CHD SNP – the effect estimates obtained
464 using the two methods were very similar (**Supplementary Table 4**).

465 Finally, to further demonstrate that the gene-smoking interaction effect in CHD at rs7178051 is
466 independent of the *CHRNA4-A3-A5* CPD locus, we conducted sensitivity analyses in the PROMIS
467 study by restricting our gene-environment interaction analysis to subjects who do not carry the minor
468 alleles of rs1051730 and rs684513 (the two SNPs associated with CPD) at the *CHRNA4-A3-A5*
469 locus. The T allele at the rs7178051 variant was associated with CHD only in never-smokers (OR:
470 0.88; P-value: 0.01) compared to a weaker and non-significant association in ever-smokers (OR:
471 0.94; P-value: 0.21) (**Supplementary Table 5**). The effect estimates obtained in each of the

categories defined by smoking status in PROMIS were similar to the effect estimates obtained in our overall meta-analyses that utilized data in all participants (**Supplementary Table 5**).

Analysis of eQTLs and regulatory features at the chr15q25.1 gene-smoking interaction locus.

We mined publicly available eQTL data from the HapMap consortium,¹¹ GTEx consortium²³ and the MuTHER consortium²⁴ as well as data from 147 HAoEC lines²⁵ to examine the association between mRNA expression of *ADAMTS7* and *CHRN* genes with CHD, CPD and gene-smoking interaction SNPs at the chr15q25.1 locus. SNP-mRNA associations with p-values <0.002 (correction for 20 tests) are presented (**Figure 5**). The top two CHD variants (rs7178051, rs11072794) are associated with reduced *ADAMTS7* expression (e.g., rs11072794 $p=6.01 \times 10^{-21}$ in MuTHER LCL, $n=850$; and rs7178051 $p=0.0029$ in HAoEC, $n=147$) but have no association with expression of *CHRN* genes in any cell or tissue examined. In contrast, the top two CPD variants (rs1051730 and rs684513) were associated with *CHRN* gene expression (e.g., rs1051730 $p=6.9 \times 10^{-7}$ for CHRNA5 in GTEx skeletal muscle and nerve tissue) but have no association with *ADAMTS7* in these cells or tissues. These findings complement conditional analyses suggesting that gene-CHD and gene-smoking interaction effects on CHD are likely mediated by *ADAMTS7* whereas the smoking behavior effect appears to be mediated through the *CHRNA3-5* gene cluster.

In analysis of data from the ENCODE project²⁶ and for human aortic tissue in NIH Roadmap Epigenomics project, *ADAMTS7* was associated with RNAseq reads and an active transcription mark, H3K36me3, whereas *CHRN* genes had low/absent RNAseq reads and were positive for repressive marks, H3K27me3 and H3K9me3 (**Supplementary Figure 8**). In HCASMC ChIPseq data, rs7178051 the top CHD and gene-smoking CHD interacting SNP, is located in a region with active regulatory marks H3K4me1 and H3K4me3 as well as transcription factor binding site for TCF21, an important HCASMC transcription factor also associated with CAD. This ChIPseq pattern was observed also in human aortic tissue (**Figure 6**). These regulatory data suggest active transcription of *ADAMTS7*, but not *CHRN* genes, in vascular cells and aortic tissue and reveal that rs7178051, the lead gene-smoking CHD interaction SNP, overlaps active transcription marks and transcription factor binding regions in HCASMC.

ADAMTS7 and *CHRNA3-A5* expression in vascular cells and their response to cigarette smoke extract

In order to explore which genes at the chr15q25.1 locus are expressed in CHD-relevant vascular cells, we performed q-PCR of *ADAMTS7* and the *CHRNA3-A5* genes in primary human

503 vascular cells and in the THP1 human monocyte cell line (**Supplementary Figure 9 & Figure 5**).
504 Whilst *ADAMTS7* mRNA was expressed abundantly in all vascular cell types, mRNA was below
505 detection or expressed at a very low level for each of the genes in the *CHRNA4-A3-A5* cluster in any
506 of these cell types (**Supplementary Figure 9**). Next, we explored the effect of cigarette smoke
507 extract on gene expression in HCASMC, a cell type of particular relevance to vascular responses to
508 cigarette smoke products^{31, 32} as well as to *ADAMTS7* vascular functions in atherosclerosis and
509 CHD.³³ In primary HCASMC, cigarette smoke extract exposure increased *ADAMTS7* mRNA levels
510 by over 2-fold (**Figure 5**) but did not affect expression of the *CHRN* genes (not shown). Thus, in
511 contrast to *CHRN* genes, *ADAMTS7* is both expressed and modulated by cigarette smoke extract in
512 CHD-relevant vascular cells providing biological support for *ADAMTS7*, but not *CHRN* genes, in the
513 gene-smoking interaction at chr15q25.1.

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DISCUSSION

We conducted a gene-environment interaction study at fifty loci associated with either CHD or smoking behavior and found evidence of effect-modification of genotype-related CHD risk by smoking-behavior at the chr.15q21.1 CHD locus. Specifically, we observed highly significant attenuation of the cardio-protective effects associated with alleles at this locus in people who smoked cigarettes. Conditional analyses identified an LD block located at the *ADAMTS7* gene that accounted for both the main effect on CHD as well as the gene-smoking interactions in CHD. Data from expression and cell studies support our genetic analysis, suggesting that the underlying mechanism relates to genotype differences in the effect of smoking on expression of *ADAMTS7* in vascular tissue.

Our findings have novel mechanistic and clinical implications. These human genomic data provide new insights into the mechanism of CHD in cigarette smokers. Identification of gene-smoking interaction at the chr15q21.1 locus suggests a specific role in smoking-related CHD for *ADAMTS7* and its substrates, vascular matrix and vascular smooth muscle cell biology more broadly. Such insights can help to prioritize translational strategies for smoking-related CHD and present opportunities to study lifestyle interventions and pharmacological strategies to lower CHD in individuals who smoke cigarettes. Thus, inhibition of *ADAMTS7* represents a novel potential therapeutic strategy for CHD that may have particular benefits in individuals who smoke cigarettes. All smokers should receive counseling for smoking cessation yet such broad public health strategies have failed to reach or impact smoking behavior in a large portion of nicotine-addicted individuals. Our data provides a human genomic context for consideration of targeting specific genetically at-risk individuals via intensified preventive strategies and development of novel pharmacological treatments.

Our study also represents a realistic strategy for performing gene-environment interaction studies using contemporary genetic data. We illustrate that identifying joint effects of genetic and lifestyle factors in CHD requires very large sample sizes, yet such analyses are biologically informative when studies are adequately powered. In this context, an important observation in our large sample is the lack of effect modification by smoking behavior on CHD at the *APOE* locus, a previously reported smoking interaction locus.¹²⁻¹⁴ This finding is consistent with a recent meta-analysis that found no evidence of effect modification by smoking for *APOE* genotypes and CHD risk.³⁴ These studies raise concerns that most prior gene-environment interactions studies in CHD have been prone to the same biases (i.e., limited statistical power and false positive associations) as

561 candidate gene studies investigating main effects in the pre-GWAS era. The present study differs
562 from previous studies by being much larger and, importantly, it includes genomic and functional
563 follow-up data supporting the plausibility of the observed gene-environment interaction.

564 *ADAMTS7* (or the A disintegrin and metalloproteinase with thrombospondin motifs-7) is a
565 member of the ADAMTS family of secreted zinc metalloproteases.^{35, 36} We previously discovered
566 and replicated genetic variation at the *ADAMTS7* locus in association with coronary atherosclerosis
567 and MI.⁷⁻⁹ Both *in vivo* and *in vitro* studies suggest that ADAMTS7 modulates VSMC phenotype
568 switching and migration and that this may be mediated via cartilage oligomeric matrix protein
569 (COMP) or thrombospondin-1 (TSP-1),^{32,33} i.e. putative ADAMTS7 substrates expressed in vascular
570 tissue. Genetic variation at *ADAMTS7*, however, has no relationship with traditional risk factors or
571 mechanistic biomarkers; hence the directional impact of *ADAMTS7* expression on CHD risk and the
572 underlying biological mechanisms have been unclear.³²

573 Our gene-smoking interaction analyses provide novel insights into the directional impact of
574 the *ADAMTS7* locus on CHD, the underlying mechanisms of CHD in smokers, and how such
575 findings ultimately might translate towards achieving health benefits in society. Our human eQTL
576 interrogations reveal that common alleles that relate to lower CHD risk at the *ADAMTS7* locus are
577 also associated with reduced *ADAMTS7* expression, implying an atherogenic role of the gene. This
578 is supported by our recent *in vivo* experimental studies; *Adamts7* deficiency protected against diet-
579 induced atherosclerosis in both the *Ldlr*^{-/-} and *ApoE*^{-/-} mouse models, reduced neointima formation
580 following arterial injury, and decreased VSMC migration *in vitro*.³³ In our smoking-stratified analyses,
581 we observed CHD protective effect which was attenuated in smokers. Thus, smoking exposure may
582 overcome the genetic effect of protective alleles that act by reducing *ADAMTS7* expression. Such a
583 possibility is supported by our HCASMC data that reveals increased *ADAMTS7* expression in
584 HCASMC exposed to cigarette smoke extract. These human genome-smoking studies are the first to
585 implicate a specific locus as causal in mediating increased risk of CHD in smokers. Additional
586 translational studies are needed to establish the precise mechanisms of atheroprotection for alleles
587 at the *ADAMTS7* locus, how cigarette smoking impacts these genetic effects, and whether deletion
588 or inhibition of ADAMTS7 *in vivo* attenuates the specific acceleration of atherosclerosis conferred by
589 cigarette smoking.

590 Strengths and limitations of our study merit consideration. This is a large study that
591 conducted gene-smoking interaction analyses in CHD by using GWAS data. We observed
592 substantial heterogeneity across study samples in our initial quality control analyses of “ever-

593 smoking” status with CHD risk. This is similar, however, to the heterogeneity reported in a recent
594 meta-analysis that pooled risk ratios from all the past prospective studies with information on
595 association of “ever-smoking” with incident CHD events.⁵ We recognize that other smoking related
596 phenotypes are important e.g., “current smoking” may have a more pronounced role than “ever-
597 smoking” in plaque rupture and thrombosis in patients with MI. We were however unable to
598 distinguish between “former” versus “current” smokers within “Ever Smokers” in our current
599 analyses; furthermore we were not able to analyze graded exposure to cigarette smoking such as
600 “pack-years”. Given the use of multiple studies and meta-analyses of data, we used only one
601 analytical approach to investigate gene-smoking interactions. This approach, however, was feasible
602 and powerful in this large-scale consortium setting. While we used a fixed effects approach in our
603 meta-analyses, a random effects meta-analysis yielded qualitatively similar results (data not shown).
604 The lack of replication is partially offset by a large sample size, consistency across study cohorts
605 and racial groups and supplemental genomic and experimental evidence supporting biological
606 plausibility. This approach is also consistent with recent recommendations³⁷ which favor use of a
607 powerful discovery experiment using all data rather than reducing power by splitting available
608 dataset for discovery and validation. While our *in vitro* studies support a role for *ADAMTS7* in the
609 gene-smoking interaction, it will be important to confirm that *Adamts7* deficiency protect against
610 cigarette-smoke acceleration of atherosclerosis in rodent models.

611 Our interaction analyses, conditional analyses, eQTL interrogations and cell studies
612 suggest that *ADAMTS7*, but not the *CHRNA4-A3-A5* gene cluster, is likely causal at 15q21.1 for
613 gene-smoking interaction effects in CHD. Yet, analyses are not definitive. Although top interacting
614 SNPs and CHD SNPs (e.g., rs7178051) were associated with *ADAMTS7*, but not *CHRNA4-A3-A5*,
615 expression in LCLs, large-scale eQTL data and allele specific expression data (e.g., via RNA
616 sequencing) are not available for vascular tissues limiting causal inference. In our small HCAEC
617 datasets, we did however find that alleles at rs7178051 associate with *ADAMTS7* expression but not
618 with any *CHRNA4-A3-A5* genes suggesting, at least in one vascular cell type, that the gene-smoking
619 interaction is mediated via *ADAMTS7*.

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Conclusions

We provide novel evidence for allelic variation exhibiting gene-smoking interaction in CHD at the chr.15q21.1 locus. The protective effect conferred by variation at this locus in never-smokers is markedly attenuated in people who are ever-smokers. Stepwise conditional analyses, gene expression data in vascular cells, eQTL interrogation, and cigarette smoke extract exposure in HCASMC suggest that *ADAMTS7* accounts for both the gene-smoking interaction in CHD and the CHD main effect on chr.15q21.1. Our findings reveal interactions of genetic variants and key lifestyle determinants in the etiology of CHD, provide new insights into the potential mechanisms of CHD in cigarette smokers, and facilitate precision medicine advances in cigarette-smoking related CHD. Our work motivates future large-scale studies investigating joint effects of genes and environment in CHD using existing complex-disease consortia datasets and genome-wide discovery approaches. This will provide opportunities to detect additional and novel loci displaying gene-environment interactions revealing genetic contexts for targeting intensive lifestyle interventions and novel therapeutics.

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1098 **Table-1.** Novel genotype-smoking interaction findings in coronary heart disease at the chromosome 15q25.1 locus

Variant	Association	allele	LD with rs7178051*	LD with rs1051730^	Never Smokers					Ever Smokers					P-value interaction
					N cases	N controls	N Total	Beta (SE)	P-value	N cases	N controls	N Total	Beta (SE)	P-value	
*rs7178051 ⁴	CHD (NPR)	T/C	-	0.22	21232	38713	59945	-0.13 (0.01)	1.30E-16	39585	40749	80334	-0.05 (.01)	2.49E-04	8.57E-05
†rs1051730 ¹⁶	SB (known)	A/G	0.22	-	20559	38198	58757	-0.04 (0.02)	0.02	38923	40371	79294	0.03 (0.01)	0.02	2.37E-04
Other variants on chr.15q25.1 with significant gene-smoking interactions on CHD															
rs7173743 ¹	CHD (Known)	C/T	0.61	0.18	21050	37955	59005	-0.11 (0.01)	2.73E-13	39044	39559	78603	-0.04 (0.01)	8.60E-04	9.29E-05
rs10083696 ²	CHD (Novel)	A/G	1.0	0.22	19721	36206	55927	-0.11 (0.02)	1.60E-12	38807	40018	78825	-0.05 (0.01)	2.72E-04	5.15E-05
rs7176187 ³	CHD (Novel)	T/C	1.0	0.24	21232	38713	59945	-0.12 (0.01)	7.02E-16	39585	40749	80334	-0.04 (0.01)	8.64E-04	6.93E-05
rs6495335 ⁵	CHD (Novel)	G/T	1.0	0.22	20144	37217	57361	-0.13 (0.02)	2.39E-15	36448	38203	74651	-0.04 (0.01)	1.69E-03	9.51E-04
rs4380028 ⁶	CHD (Known)	T/C	1	0.22	21232	38713	59945	-0.12 (0.01)	2.20E-15	39585	40749	80334	-0.04 (.01)	1.03E-03	5.44E-04
rs3825807 ⁷	CHD (Known)	G/A	0.52	0.43	17137	28633	45771	-0.09 (0.02)	2.82E-08	30071	29014	59086	-0.03 (0.01)	0.04	2.6E-03
rs3813565 ⁸	CHD (NPR)	T/G	0.43	0.56	19466	35830	55296	-0.08 (0.02)	5.08E-07	36642	37759	74401	-0.01 (0.01)	0.42	3.05E-04
rs11638490 ⁹	CHD (NPR)	T/C	0.44	0.51	20465	37897	58362	-0.08 (0.01)	6.90E-08	38533	39690	78223	-0.01 (0.01)	0.28	2.25E-04
rs11072791 ¹¹	CHD (NPR)	A/C	0.44	0.51	19289	35944	55233	-0.08 (0.02)	2.83E-07	35245	36635	71880	-.005 (0.01)	0.68	1.06E-04
rs922692 ¹²	CHD (NPR)	A/C	0.44	0.50	20559	38198	58757	-0.08 (0.01)	2.81E-07	38923	40371	79294	-0.01 (0.01)	0.29	2.75E-04
rs11638372 ¹³	CHD (NPR)	T/C	0.44	0.50	21232	38713	59945	-0.08 (0.01)	6.92E-08	39585	40749	80334	-0.01 (0.01)	0.23	3.16E-04
rs4887077 ¹⁴	CHD (NPR)	T/C	0.44	0.50	21232	38713	59945	-0.08 (0.01)	4.71E-08	39585	40749	80334	-0.02 (0.01)	0.20	3.92E-05
rs12899135 ¹⁵	CHD (NPR)	G/A	0.39	0.56	20377	37440	57817	-0.07 (0.02)	3.97E-06	38382	39181	77563	-0.01 (0.01)	0.58	4.54E-04
rs684513 ¹⁸	SB (Known)	C/G	0.01	0.10	12517	21054	33572	-0.01 (0.02)	0.67	24641	24487	49129	0.03 (0.02)	0.18	0.08
rs2036527 ¹⁹	SB (Known)	A/G	0.17	0.90	20559	38198	58757	-0.04 (0.02)	0.02	38923	40371	79294	0.03 (0.01)	0.02	2.14E-04
rs10519203 ²⁰	CHD (NPR)	G/A	0.19	0.93	21232	38713	59945	-0.04 (0.01)	5.93E-03	39585	40749	80334	0.03 (0.01)	0.03	1.27E-04
rs8034191 ²¹	SB (Known)	C/T	0.19	1.0	19251	32131	51382	-0.05 (0.02)	2.62E-03	34925	34047	68972	0.02 (0.01)	0.06	3.91E-05

1099 CHD = coronary heart disease; SB = smoking behavior; NPR: Not a previously reported variant with disease risk
1100 *lead variant in association with CHD in our dataset; † lead variant in association with SB
1101 ¹⁻²¹each number refers to the physical location of the variant in figure-

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1111 **Figure Legends**

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1113 **Figure 1.** (a) Regional association analyses at the chromosome 15q25.1 locus in association with
1114 CHD risk stratified by smoking status. Association P-values for genetic variants with CHD risk in
1115 “never-smokers” (green squares) and “ever-smokers” (red triangles). (b) Longitudinal bars represent
1116 gene-smoking CHD interaction P-values at the chromosome 15q25.1 locus; bars in blue are P-
1117 values for variants listed in Table-1 and each variant has been assigned a unique identification
1118 number based on its physical location; (c) LD-blocks at the 15q25.1 locus visualized through
1119 HAPLOVIEW using LD estimates in the HapMAP-2 CEU reference population.

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1121 **Figure 2.** Several variants at chromosome 15q21.1 have stronger effects on CHD risk in “never-
1122 smokers” compared to “ever-smokers”. Variants with the strongest interaction P-value are displayed.

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1124 **Figure 3.** Step-wise conditional analysis of genetic variation at the chromosome 15q21.1 locus with
1125 CHD (red triangles) and smoking behavior (cigarettes per day, CPD; grey circles). At the
1126 chromosome 15q21.1 locus, analyses adjusted for rs7178051 and rs11072794 completely
1127 attenuated the gene-CHD associations whereas gene-smoking remained unchanged. Analyses
1128 adjusted for rs1051730 and rs684513 completely attenuated the gene-smoking associations
1129 whereas gene-CHD effect remained unchanged.

1130 **Figure 4.** Analyses mutually adjusted for rs7178051, rs11072794, rs1051730 and rs684513 at
1131 15q21.1 on CHD and smoking behavior; gene-CHD interaction analyses were only found significant
1132 for rs7178051. Analyses on the left panel show associations of rs7178051, rs11072794, rs1051730
1133 and rs684513 with CHD risk mutually adjusted for each other. Analyses on the right panel show
1134 associations of rs7178051, rs11072794, rs1051730 and rs684513 with smoking behavior mutually
1135 adjusted for each other.

1136 **Figure 5.** (a) *ADAMTS7* and *CHRNA3-4-5* mRNA levels were measured in HCASMC. Cells were
1137 cultured to confluence, total RNA was extracted and cDNA generated. q-PCR was performed for
1138 *ACTB*, *GAPDH*, *TBP*, *ADAMTS7*, *CHRNA3*, *CHRNA4*, *CHRNA5* (95°C 15s, 60°C 1min). Delta Cts
1139 were calculated as follows: $(Ct_{ACTB} + Ct_{GAPDH} + Ct_{TBP})/3 - Ct_{TARGET\ GENE}$. Fold changes are derived
1140 from delta delta Cts based on formula $FC = 2^{-\Delta\Delta Ct}$. (b) Confluent HCASMC were exposed to cigarette
1141 smoke extract. Serum starved (x24 hrs.) confluent HCASMC were treated with 0.5% or 1.0%
1142 cigarette smoke extract (v/v) for 4, 12, and 24 hrs. in serum reduced conditions (0.5% FBS in
1143 DMEM). Total RNA was extracted, cDNA generated preparation and q-PCR performed for

1144 *ADAMTS7* by Taqman and normalized to *GAPDH*. The Average Ct for *ADAMTS7* at baseline was
1145 28.25. Results were presented as means \pm SEM, and data were analyzed using Student's t-Test. (c)
1146 expression and eQTL Data from the GTEx consortium, the HapMap consortium (restricted to
1147 European populations), the Multiple Tissue Human Expression Resource (MuTHER) and in 147
1148 donor HAoEC lines. Association of the independent lead variants identified in our conditional
1149 analyses with expression of *ADAMTS7* and genes in the *CHRNA4-A3-A5* cluster. A P-value
1150 threshold of 0.002 was set to account for multiple testing involved in the eQTL analyses.

1151 **Figure 6.** Genome browser view of regulatory features at rs7178051 on Chr15q21.1. ChIP-seq
1152 experiments were performed on confluent HCASMC for TCF21, Jun, JunD, CEBP and H3K4me1,
1153 H3K27me3, H3K27ac. DNaseI hypersensitivity data for human AoSMC were acquired from the
1154 ENCODE project. Human aortic tissue H3K4me1, H3K9me3, H3K27me3, and H3K36me3 ChIP-seq
1155 data were acquired from the NIH Roadmap Epigenomics Project. HCASMC = human coronary
1156 artery smooth muscle cells; AoSMC = human aortic smooth muscle cells.

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